

Hybrid forms of the *Culex pipiens* Complex (Diptera: Culicidae): New records in La Pampa Province, Argentina

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ABSTRACT: The present investigation revealed the existence of hybrids belonging to the *Culex pipiens* Complex in four localities of La Pampa Province, Argentina, thus expanding the distribution of the hybrid form south to the northeast of that province. The identification was based on characters of the male genitalia and the siphonal index of the fourth-instar larval exuviae. A summary of the geographical distribution of species of *Culex pipiens* complex in Argentina is also presented here.

The *Culex pipiens* Complex is widely studied because of its medical and veterinary relevance as a vector of pathogens that cause various diseases to humans as well as animals (*e. g.*, encephalitis arboviruses, filariasis, and avian malaria; Mitchell *et al.* 1985; Díaz *et al.* 2006; Vezzani *et al.* 2006). Members of this complex can be differentiated by certain ecophysiologic characteristics such as the production of the first egg raft without previously feeding on blood (autogeny), the ability to mate in small spaces (stenogamy), and the preference for open spaces to mate (eurygamy); molecular-genetic techniques (*e. g.*, gene mapping by PCR); and morphometric indices (Vinogradova 2003; Vinogradova *et al.* 2007). Among the latter are the siphonal index (S. I.) and the DV/D ratio. The S. I. is the ratio between the length of the siphon, (measured as the distance from the base to the apex of the siphon, on the dorsal surface, excluding the spiracular apparatus), and the basal width of the siphon, (measured at right angles to the longitudinal axis of the siphon) (Brogdon, 1981, 1984). The DV/D ratio, as applied to the male genitalia, is the ratio of the distance between the ends of the dorsal and ventral arms of lateral plate of the phallosome on one side and the distance between the two dorsal arms (Barr, 1957).

In Argentina, *Culex pipiens* Complex is represented by: *Culex (Culex) quinquefasciatus* Say, *Culex (Culex) pipiens* Linnaeus, and hybrids between the two species. The range of its distribution, as well as of its hybrids, is not entirely clear. *Culex quinquefasciatus* occurs in the central and northern regions, whereas *Cx. pipiens* is found from central and southern Argentina (south of Santa Cruz Province) (Duret, 1953; Mitchell *et al.* 1984; Mitchell and Darsie, 1985). Therefore, distribution of both species overlaps in the central region (Mitchell *et al.* 1984; Brewer *et al.* 1987; Almirón *et al.* 1995; Diez *et al.* 2011). With respect to the hybrids, Brewer *et al.* (1987) recorded their presence in Quebracho Flojo Village, Córdoba capital city, and the city of Cruz del Eje, all in Córdoba Province, situated between

latitudes 30° S and 32° S. In addition, Almirón *et al.* (1995) recorded hybrids in the city of Rosario, Santa Fé Province, latitude 36°13'37" S; and Morais *et al.* (2010) did it in La Plata, Buenos Aires Province at a latitude of 35° S. (Fig.1)

Culex quinquefasciatus (Carpintero and Leguizamón, 2004) and *Cx. pipiens* (Diez *et al.* 2011) were also recorded in La Pampa Province, although no hybrids were found there. The objectives of this study were: to assess the presence of hybrids of *Culex pipiens* and *Culex quinquefasciatus* in the northeastern section of La Pampa Province; and to clarify the distribution of species of *Culex pipiens* Complex in Argentina.

The areas sampled in La Pampa, during February and March 2010, were Winifreda (36°13'37" S, 64°13'58" W), Eduardo Castex (35°54'57" S, 64°17'44" W), General Pico (35°39'42" S, 63°44'49" W), and Realicó (35°1'59" S, 64°14'47" W). These are located within the phytogeographic region of Espinal, it featuring a semiarid to semihumid and temperate climate, with an annual rainfall of 600 to 800 mm (Cano *et al.* 1980).

In each locality, we investigated two pneumatic-tire yards and the local cemetery. In the first area (Winifreda), 322 containers (glass, metal, plastic, and ceramic jars of from 0.5 to 2 liters) were surveyed, among which only 7 samples were positive. Larvae and pupae were taken from the containers and transported to the laboratory. Similar numbers of positive samples were taken from the other sampling sites to maintain a constant sampling volume along the entire experiment. From the several samples from used-tire warehouses, 5 were found positive as regards the presence of immature *Culex pipiens* Complex mosquitoes.

The immature mosquitoes were transported to the Biología de Invertebrados II laboratory at UNLPam. Larvae and pupae from each location were both kept in 1,500 ml containers, containing 750 ml of chlorine-free water at 25 ± 4 °C in a 14:10-h. light-dark photoperiod. Larvae were fed 200 mg commercial chicken food every 4 days up to the

pupae stage. During this period, the fourth- instar larval exuviae were separated to assess the S. I. under a Kyowa stereoscopic microscopic at a 72X magnification. Once emerged, the adults were sugar-fed on raisins along three days. The distance between the ends of the dorsal and ventral arms of lateral plate of the phallosome on one side, and between the two dorsal arms were measured under a Nikon YS100 binocular microscope at a 100X magnification with the aid of an ocular micrometer. Both the siphons and the genitalia were placed on microscope slides immersed in a drop of 80% ethanol without cover slips in order to

avoid distortions. Specimens with S. I. values of 4.30 or higher were identified as *Cx. pipiens*; those with values of 3.40 or lower were identified as *Cx. quinquefasciatus*; and those with S. I. values >3.41 but <4.29 were considered to be hybrids (Brogdon, 1981, 1984). Specimens with DV/D ratios of 0.20 or lower were identified as *Cx. pipiens*; those with ratio of 0.40 or higher as *Cx. quinquefasciatus*; and those with ratio >0.21 but <0.39 as hybrids (Barr, 1957).

A total of 845 *Culex pipiens* Complex larvae were collected, 368 of which were males and 477 females. All siphons of fourth-instar larval exuviae were measured and

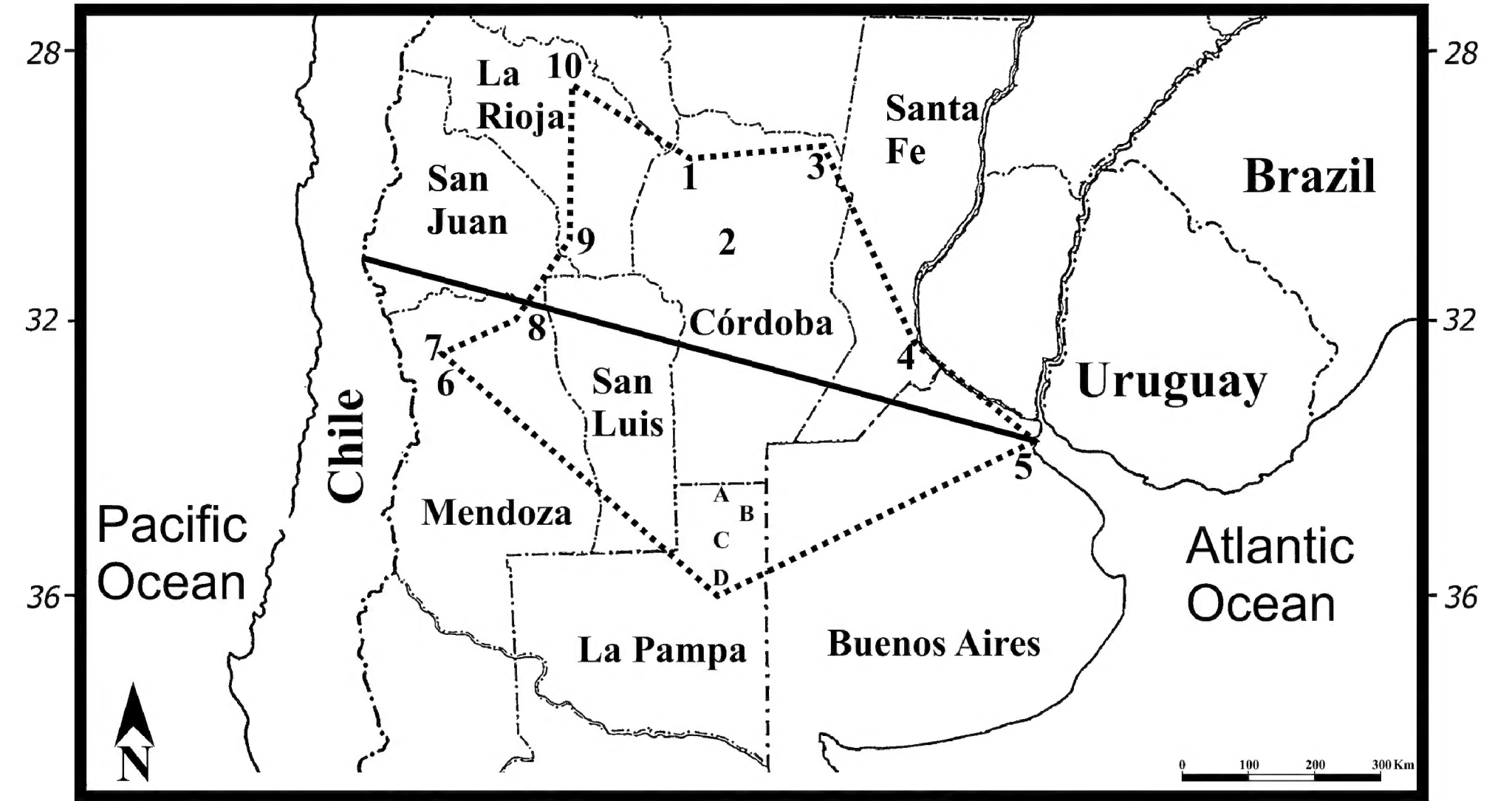


FIGURE 1. Presence of hybrids of *Culex quinquefasciatus* and *Culex pipiens* in Argentina. La Pampa Province: A: Realicó, B: General Pico, C: Eduardo Castex, D: Winifreda. Córdoba Province: 1. Huascha, 2. Córdoba City, 3. Quebracho Flojo, 4. Rosario, 5. La Plata. Presence of species of *Culex pipiens* Complex in the central west region: 6: Mendoza, 7: Uspallata, 8: Lavalle, 9: Chepes, 10: Aimogasta. Overlap distribution of *Culex quinquefasciatus* and *Culex pipiens* is marked with a dotted line. Limit of the distribution between *Culex quinquefasciatus* and *Culex pipiens* proposed by Mitchell and Darsie (1985) is marked by a solid line. See details in the text.

TABLE 1. Presence of hybrids of *Culex quinquefasciatus* and *Culex pipiens*, in four localities of La Pampa Province.

Localities		Identified by DV/D				Nº of females not identified	Identified by S. I.				Nº flower vases with larvae	Nº tires with larvae	
		Cx. pipins	Cx. quinquefasciatus	Hybrids	Total		Cx. pipiens	Cx. quinquefasciatus	Hybrids	Total		Tire yard Nº I	Tire yard Nº II
Winifreda	n	38	10	1	49	196	171	46	28	245	7	0	5
	%	77,6%	20,4%	2%	100%		69,8%	18,8%	11,4%	100%			
Castex	n	84	22	4	110	122	110	52	70	232	7	4	5
	%	76,4%	20%	3,6%	100%		47,4%	22,4%	30,2%	100%			
General Pico	n	1	35	12	48	81	25	76	28	129	5	5	3
	%	2,1%	72,9%	25%	100%		19,4%	58,9%	21,7%	100%			
Realicó	n	59	94	8	161	78	78	112	49	239	7	5	5
	%	36,6%	58,4%	5%	100%		32,6%	46,9%	20,5%	100%			

the S. I. was assessed for species identification. We likewise estimated the DV/D-ratio for the 368 males.

On the basis of the present findings, the distribution of hybrids of the *Culex pipiens* Complex in Republic of Argentina now extends to the latitude 36°13'37" S, corresponding to the above detections within Winifreda in La Pampa Province. From these results and registers from the published literature, hybrids of *Culex pipiens* Complex occur between latitudes 30° 36' S (Huascha, Córdoba Province, Brewer et al. 1987) and 36°13' S (Winifreda, La Pampa Province), and between longitudes 57° 57' W (La Plata, Buenos Aires Province; Morais et al. 2010) and 64°48' W (Cruz del Eje, Córdoba Province, Brewer et al. 1987). Results of the present study suggest that the northeastern portion of La Pampa Province is an area of hybridization similar to southern Santa Fe Province (Almirón et al. 1995) and central and northern Córdoba Province (Brewer et al. 1987).

Records of *Cx. pipiens* in Chepes (31°21'00" S, 66°37'00" W), La Rioja Province (Visintin et al. 2010); Uspallata, Las Toscas (32°53'26" S, 68°48'56" W), Mendoza Province (Mitchell et al. 1984); and Winifreda (36°13'37" S, 63°16'52" W), La Pampa Province (Diez et al. 2011) along with those of *Cx. quinquefasciatus* in the cities of Mendoza (32°53'10" S, 68°50'38" W) (Rossi et al. 2006) and Lavalley (32°52'44" S, 58°48'10" W) (Rossi, Pers. Comm.), both Mendoza Province; Aimogasta (28°32'17" S, 66°48'42" W) (Duret, 1953), La Rioja Province; and Toay (31°21'00" S, 66°37'00" W) (Carpintero and Leguizamón 2004) and Winifreda (36°13'37" S, 63°16'52" W) (Diez et al. 2011) both La Pampa Province, permit the inference that the overlapping area for both species lies between the latitudes 31° and 36° S in Argentina (Figure 1).

The distribution of *Cx. quinquefasciatus* in Argentina therefore comprises the following provinces: Buenos Aires, Chaco, Corrientes, Formosa, Misiones, Salta, Santa Fé, and Tucumán (Duret, 1951); Catamarca, Entre Ríos, Jujuy, La Rioja, and Santiago del Estero (Duret, 1953); Córdoba (Mitchell and Darsie, 1985); La Pampa (Carpintero and Leguizamón 2004; Diez et al. 2011); and Mendoza (Rossi et al. 2006). *Culex pipiens* has been registered in the following provinces: Buenos Aires (Duret, 1951); Chubut and Mendoza (Mitchell et al. 1984); Córdoba, San Juan, and San Luis (Mitchell and Darsie, 1985); Corrientes (Rossi, 1995); La Pampa (Diez et al. 2011); La Rioja (Visintin et al. 2010); and Neuquén, Rio Negro, and Santa Cruz (Bachmann and Bejarano, 1960).

Relevance of the present study to human and animal health can be easily justified as species of the *Culex pipiens* Complex may be vectors of the St. Louis encephalitis virus (SLEV). Since previous studies have reported the presence of specimens of *Cx. quinquefasciatus* infected with SLEV in Córdoba and Santa Fe Provinces (Mitchell et al. 1985; Diaz et al. 2006), a better knowledge of the distribution range of species of the Complex throughout Argentina becomes a critical health issue.

LITERATURE CITED

- Almirón, W.R., S.G. Humeres and C.N. Gardenal. 1995. Distribution and hybridization between *Culex pipiens* and *Culex quinquefasciatus* (Diptera: Culicidae) in Argentina. *Memórias do Instituto Oswaldo Cruz*. 90: 469-473.
- Bachmann, A.O. and J. F.R. Bejarano. 1960. Dispersion de mosquitos en la Patagonia. *Neotropica* 6:70-71.
- Barr, A.R. 1957. The distribution of *Culex p. pipiens* and *C. p. quinquefasciatus* in North America. *The American Journal of Tropical Medicine and Hygiene* 6: 153-165.
- Brewer, M.M., L. Buffa and W.R. Almirón. 1987. *Culex pipiens quinquefasciatus* y *Culex pipiens pipiens* (Dip.: Culicidae) en Córdoba, Argentina. *Revista Peruana de Entomología* 29: 69-72.
- Brogdon, W.G. 1981. Use of the siphonal index to separate *Culex pipiens* subspecies and hybrids. *Mosquito Systematics* 3: 129-137.
- Brogdon, W.G. 1984. The siphonal index. A method for evaluating *Culex pipiens* subspecies and intermediates. *Mosquito Systematics* 16: 144-152.
- Cano, E., G. Casagrande, H.A. Conti, J.C. Salazar Lea Plaza, C.A. Peña Zubiarte, D. Maldonado Pinedo, H. Martínez, R. Hevia, C.O. Scoppa, B. Fernández, M.A. Montes. 1980. *Inventario Integrado de los Recursos Naturales de La Pampa*. Buenos Aires: Instituto Salesiano de Artes Gráficas. 493 p.
- Carpintero, D. J. and M. N. Leguizamón. 2004. Description of a new *Culex* (*Culex*) species (Diptera: Culicidae) from La Pampa province, Argentina, and a preliminary list of the mosquitoes of the province. *Studia Dipterologica*.11: 501-503.
- Diaz, L., V. Ré, W.R. Almirón, A. Farias, A. Vazquez, M.P. Sanchez-Seco, J. Aguilar, L. Spinsanti, B. Konigheim, A. Visintin, J. Garcias, M.A. Morales, A. Tenorio and M. Contigiani. 2006. Genotype III Saint Louis encephalitis virus outbreak, Argentina, 2005. *Emerging Infectious Diseases* 12: 1752-1754.
- Diez, F., V.J. Breseer, E.M. Quirán and G.C. Rossi. 2011. Nuevos registros de mosquitos (Diptera: Culicidae) en la provincia de La Pampa, Argentina. *Revista Sociedad Entomologica Argentina* 70: 347-349.
- Duret, J.P. 1951. Contribución al conocimiento de la distribución geográfica de los culicidos argentinos. (Diptera-Culicidae). Parte II. *Revista de la Sanidad Militar Argentina* 50: 64-72.
- Duret, J.P. 1953. Notas sobre *Culex* argentinos (Diptera-Culicidae). *Revista de la Sanidad Militar Argentina*. 52: 272-278.
- Mitchell, C.J., R.F. Jr. Darsie and T.P. Monath. 1984. Occurrence of autogenous *Culex pipiens* Linnaeus, 1758, (Diptera: Culicidae) in Argentina and notes on distribution of the Complex. *Mosquito Systematics* 16: 308-316.
- Mitchell, C. J. and R. F. Darsie. 1985. The mosquitoes of Argentina. Part II. Geographic distribution and bibliography (Diptera: Culicidae). *Mosquito Systematics*. 17: 279-360.
- Mitchell, C.J., T.P. Monath, M. Sabattini, C. Cropp, J. Daffner, F. Calisher and H. Christensen. 1985. Arbovirus investigations in Argentina. II, Arthropod collections and virus isolations from mosquitos. *The American Journal of Tropical Medicine and Hygiene* 34: 945-955.
- Morais, S.A., S., C. Moratore, L. Suesdek, and M.T. Marrelli. 2010. Genetic-morphometric variation in *Culex quinquefasciatus* from Brazil and La Plata, Argentina. *Memórias do Instituto Oswaldo Cruz*. 105: 672-676.
- Rossi, G.C. 1995. Culicidos nuevos para las provincias de Entre Ríos y Corrientes. *Revista Sociedad Entomologica Argentina*. 54: 97-98.
- Rossi, G.C., E.A. Lestani and J.M. D'oria. 2006. Nuevos registros y distribución de mosquitos de la Argentina (Diptera: Culicidae). *Revista Sociedad Entomologica Argentina* 65: 51-56.
- Vezzani, D. and A. E. Carbajo. 2006. Spatial and temporal transmission risk of *Dirofilaria immitis* in Argentina. *International Journal for Parasitology* 36: 1463-1472.
- Visintin, A.M., M. Laurito, M. Stein, P. Ramirez, G. Molina, P.R. Lorenzo, and W.R. Almirón. 2010. Two new mosquito species and six new provincial records in Argentina. *Journal of the American Mosquito Control Association* 26: 91-94.
- Vinogradova, E.B. 2003. Ecophysiological and morphological variations in mosquitoes of the *Culex pipiens* complex (Diptera: Culicidae). *Acta Societatis Zoologicae Bohemicae* 67: 41-50.
- Vinogradova, E.B., E.V. Shaikevich and A.V. Ivanitsky. 2007. A study of the distribution of the *Culex pipiens* complex (Insecta: Diptera: Culicidae) mosquitoes in the European part of Russia by molecular methods of identification. *Comparative Cytogenetics* 1: 129-138.

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